ALLERGEN FORMULATION

This invention relates to novel formulations for use in desensitisation therapy of allergy sufferers.

It is known that desensitisation therapy results in a changed immunological response specific for the allergens administered. Such changes are considered to be responsible for the beneficial effects of the treatment and amelioration of the symptoms of allergy.

The immunological changes responsible for benefit are not entirely understood. Although a raised allergen specific IgG antibody response is considered to be a desirable outcome of therapy, it is now believed that certain changes in the allergen specific T cell (T lymphocyte) response are more important.

Two subclasses of T cell, TH1-like and TH2-like interact with one another via various messenger molecules. In an allergic subject it appears that there is a greater allergen specific TH2 than a TH1 activity. This can lead to a high allergen specific IgE antibody level and greater eosinophil activity. These are two important components of the allergic syndrome.

A change in the above situation to one where there is greater allergen specific TH1 rather than TH2 activity is thought to be an important component of immunotherapy leading to a clinical benefit.

GB-A-1 377 074 describes a process for preparing coprecipitates of tyrosine having an allergen dispersed therein.

GB-A-1 492 973 describes a process for preparing coprecipitates of tyrosine having a modified allergen dispersed therein. The allergen has been modified by treatment with an agent, such as glutaraldehyde, which causes intra-molecular cross-linking and reduces the allergenicity of the product relative to the unmodified allergen.

3 De-O-acylated monophosphoryl lipid A (hereinafter 3-DMPL or "MPL") is known from ~~GB 2220211 (Ribi).~~ Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International ~~Patent Application No. 92/116556.~~ 3-DMPL is an example of a substance that can enhance the TH1 over TH2 directing properties of administered allergens.

According to the present invention there is provided a pharmaceutical composition comprising tyrosine, an optionally modified allergen, and 3-DMPL. Typically, the allergen is coated with and /or adsorbed onto tyrosine, for example by co-precipitation or mixing.

The 3-DMPL can be mixed with the other components of the composition prior to administration. Alternatively it can be formulated together with the other components during manufacture of the product. Alternatively, it can be administered at a different site or time

than the other components. Administration can be by a number of routes including parenteral and enteral.

A further aspect of the invention thus provides a method of treating a patient who is susceptible to allergy, which method comprises administering to the said patient an effective amount of tyrosine, an optionally modified allergen, and 3-DMPL.

A further aspect of the invention provides use of tyrosine, an optionally modified allergen, and 3-DMPL, in the preparation of a medicament for use in the prevention or treatment of allergy.

The allergen may be derived from any allergy causing substance, such as a pollen (e.g. ragweed or birch pollen), food, insect venom, mould, animal fur, or house dust mite (*D. farinae* or *D. pteronyssinus*). As used herein, "allergen" includes a mixture of allergens which may be from a single source or more than one source. The term "allergen" also includes peptides containing one or more epitopes of an allergen, such as allergen fragments, prepared by total synthesis, by enzymatic degradation of allergens, or by other means.

The allergen is optionally modified by reaction with a cross-linking agent such as a dialdehyde, more particularly glutaraldehyde.

A further aspect of the invention provides a process for the preparation of a pharmaceutical composition in accordance with the invention, which process comprises (a) (optionally) modifying an allergen by reaction with a cross-linking agent, (b) mixing an aqueous solution of the optionally modified allergen with a solution of tyrosine in a strong aqueous acid, (c) neutralising the mixture of solutions, thereby co-precipitating tyrosine and modified allergen, (d) mixing the product with 3-DMPL, and (e) optionally, adding a physiologically acceptable carrier.

Suitable physiologically acceptable carriers include phenol-saline and sterile water.

Typically, the allergen is modified by treatment with a dialdehyde such as glutaraldehyde, in aqueous solution at a pH of between 5 and 10, typically about 7, and a temperature of between 0 and 100 °C, more usually between 4 and 37 °C, for up to 10 hours, for example about two hours at room temperature. The ratio of allergen to glutaraldehyde is typically in the range 50:1 to 2:1, for example about 10:1.

The intermediate can be freeze dried or used in the next stage.

A solution of the modified allergen, typically at pH 7±1, obtained either as the reaction mixture from the cross-linking process or from the solvation of a solid, is then mixed with a solution of tyrosine in a strong aqueous acid. The strong acid is usually an inorganic acid, preferably hydrochloric acid. The solution of allergen used in this step typically contains between 0.1 µg/ml and 1000 µg/ml allergen protein, for example about 400µg/ml. The ratio of allergen: tyrosine in the mixture is typically in the range $1:4 \times 10^5$ to $1:1 \times 10^2$ w/w.

The resulting mixture of solutions of allergen and tyrosine is neutralised. By neutralisation is meant an adjustment of pH to a value within the range 4.0 to 7.5. It is important that, at no time, or at least at no prolonged time, during the neutralisation does the pH of the solution rise appreciably above 7.5. This condition can be met by vigorous stirring of the solution and by the use only of the required amount of base, if desired. Various buffering agents can usefully be added to the solutions of allergen to assist in pH control during the mixing and neutralising stages.

A particularly useful method of carrying out the neutralisation is for separate streams of the solution of tyrosine in acid and the neutralising base to be run into the solution of allergen. The rates of flow of the added solutions are controlled by pH-state, that is by equipment which regulates the flow of one or both of the solutions so that the pH of the reaction mixture remains substantially constant at a predetermined level. We have found that optimum results are usually obtained by pH control within the range 6.5 to 7.5 though the precise pH may vary according to the nature of the allergen.

The result of the neutralisation is the immediate precipitation of the tyrosine, within and/or upon which the solution of allergen is occluded and/or adsorbed. After the precipitation the mixture is either washed immediately or allowed to stand for a period of from a few hours to a day or two prior to washing.

The resulting precipitate may be removed from the solution by centrifugation or filtration and washed, e.g. with phenol-saline, before being resuspended in a physiologically-acceptable carrier such as phenol-saline, or sterile water, to produce an injectable composition suitable for use in desensitisation therapy in combination with 3-DMPL.

MPL which has been dissolved by the method described in Preparation 3 below or by sonication can be diluted by various means prior to its addition to tyrosine adsorbates of allergens or modifed allergens. The preparation of MPL is initially made at a concentration of typically between 0.5mg per ml and 4 mg per ml, for example 1 mg per ml. It can then be diluted to a concentration of between 500 µg per ml and 20 µg per ml, preferably about 100 µg per ml. This dilution can be made in pure water, or in an aqueous glycerol solution containing between 1% and 4 %, preferably 2 %, glycerol. Such dilutions can then be added to a suspension of the tyrosine adsorbate prepared as described above. For convenience, the concentration of the MPL solution and the tyrosine adsorbate suspension respectively may be selected such that approximately equal volumes of each are admixed to obtain the final product for injection. A typical final product contains about 100µg per ml of allergen and about 250µg per ml of MPL.

The following Example illustrates the present invention:

Preparation 1

A neutral solution of approximately 0.5 mg/ml grass pollen extract which had been partially purified by dialysis or fractionation was chemically modified by the addition of an equal volume of 0.25% w/v glutaraldehyde and the mixture stirred for approximately 2 hours at room temperature. To the above mixture was added phosphate buffer solution at a pH of 7 ±1. The allergen solution was co-precipitated with tyrosine by the simultaneous addition of one volume of L-tyrosine in HCl (prepared by dissolving 24g L-tyrosine to 100ml with 3.8M HCl) and one volume of 3.2M NaOH, to four volumes of allergen solution, with vigorous agitation. The suspension so formed was centrifuged, washed repeatedly with buffered saline to remove contaminants and resuspended to the original volume in buffered saline pH6 ±1.

3-DMPL suitable for coadministration with the above formulation was prepared as described in Preparation 3 below.

Preparation 2

Eight mg of ovalbumin (XOA) were dissolved by mixing in 20ml of EVANS solution. Next 6.9ml of phosphate buffer were added with mixing. The solution was placed in a 100ml beaker containing a magnetic stir bar. While mixing using a magnetic stirrer, 6.9ml of 3.2N NaOH and 6.9ml of 3.8N HCL containing 24% W/V-tyrosine were added simultaneously, dropwise, over a period of 5 min. to form a precipitate. The mixture was allowed to stir for an additional 5 min. and then transferred to a 50ml centrifuge tube and centrifuged for 10 min. at 2500 rpm. After centrifugation the supernatant was decanted and the pelleted precipitate resuspended in 40ml of phosphate buffer. The mixture was centrifuged for 5 min. at 2500 rpm. After centrifugation the supernatant was decanted and the precipitate resuspended in 40ml of phosphate buffer. The mixture was centrifuged for 5 min. at 2500 rpm. After centrifugation the supernatant was decanted and the pelleted precipitate resuspended in 40ml of phosphate buffer saline, pH7.2, containing 0.4% V/V glycerol and 0.01% W/V thimerosal as a preservative. The final product contained approximately 40mg/ml of tyrosine adsorbate. Assuming 100% binding of the XOA to the tyrosine adsorbate the XAO was at 200µg/ml in the final product. The XOA-tyrosine adsorbate was stored at 4°C until needed.

Preparation 3

A 4 mg/ml solution of 1,2-dipalmitoyl-SN-glycero-3-phospho choline (DPPC) in absolute ethanol was prepared. For each 1.0mg of MPL®-TEA salt to be solubilized, 27µl of DPPC were added to dissolve the MPL®. The ethanol was removed by blowing a stream of N_2 gently into the vial. Next 1.0ml of pyrogen-free water for injection was added for each mg of MPL® in the dried MPL® /DPPC mixture. The solution was sonicated in a bath sonicator at 60-70°C until clear. The MPL®/DPPC solution was then filter sterilized by filtration through a SFCA 290-4520 Nalgene 0.2µm filter. The MPL®/DPPC solution was aseptically dispensed at 1.0mg/ml into depyrogenated vials, labelled MPL®-AF, and stored at 4°C.

Biological activity

TH1 inducing activity in mice can be equated with the production of IgG2a and IgG2b antibodies and the TH2 inducing activity with the production of IgG1 antibodies and IgE antibodies.

Therefore, as an example, an experiment was carried out in mice to demonstrate the profiles of the allergen specific antibodies to an exemplar allergen ovalbumen (XOA) which is a well-known food allergen derived from chicken eggs. It was confirmed that a formulation consisting of MPL + XOA+tyrosine stimulated a more advantageous antibody profile than MPL + XOA, XOA+tyrosine or XOA alone.

Groups of 8 BALB/c female mice, 6-8 weeks of age, were injected subcutaneously in the inguinal area with 0.2ml of one of the following vaccines:

XOA+Tyrosine: The XOA tyrosine adsorbate prepared in Preparation 2 above was diluted with an equal volume of phosphate buffered saline within 30 min. prior to injection.

XOA+Tyrosine+MPL: The XOA tyrosine adsorbate prepared in Preparation 2 above was diluted with an equal volume of MPL®-AF at 500µg/ml in phosphate buffered saline within 30 min. prior to injection.

XOA+MPL: XOA was dissolved in phosphate buffered saline at 200µg/ml and diluted with an equal volume of MPL®-AF at 500µg/ml in phosphate buffered saline within 30 min. prior to injection.

XOA Alone: XOA was dissolved at 200µg/ml in phosphate buffered saline and diluted with an equal volume of phosphate buffered saline.

Twenty-one days later the four groups of mice were boosted with 0.2ml of freshly prepared vaccines. Fourteen days following the booster the mice were bled and the sera separated and stored at -70°C until assay.

The sera were assayed by conventional ELISA technique using horseradish conjugated goat anti-mouse IgG_1, IgG_{2a}, and IgG_{2b} antibodies purchased from Southern Biotechnology, Inc. (Birmingham, AL) and used according to the manufacturer's instruction. The IgG_1, IgG_{2a}, and IgG_{2b} titers represent the reciprocal serum dilution giving a reading of >0.1 OD units at A_{490}. The serum IgE levels were measured using an anti-IgE capture ELISA followed by the use of a biotinylated ovalbumin probe. Binding was measured following the addition of a horseradish conjugated strepavidin preparation. The results are reported as OD units at A_{490}.

RESULTS

Formulation	IgG_1 titre	IgG_{2a} titre	IgG_{2b} titre	IgE OD at 1/10 Diltn
XOA + Tyrosine	102400	100	200	0.213
XOA + Tyrosine + MPL	409600	102400	102400	0.104
XOA + MPL	102400	200	400	0.218
XOA alone	6400	<100	<100	0.235
Normal Mouse Serum Values	<100	<100	<100	0.095

Of particular importance is the fact that the combination of allergen + tyrosine + MPL induces less allergen specific IgE antibody than the other combinations. Furthermore, the ratio of IgG2a or IgG2b to IgG1 antibodies is greater and consistent with the highest levels of the two former antibody isotypes seen in the experiment in the mice given allergen+tyrosine+MPL than in any other group of mice. This is indicative of a better ratio of TH1 cell induction over TH2 cell induction in this group compared with that induced in the other groups of mice.